IN-VITRO MEASUREMENT OF IRON CONCENTRATION IN HUMAN HEPATIC TISSUE BY MAGNETIC INDUCTION METHODS

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Abstract. **This article studies the feasibility of a magnetic induction (MI) based system to measure the concentration of paramagnetic material in biological tissues. Our final objective is the measurement of hepatic iron overload with a non-invasive method. We have used an analytical expression, for a disc of conducting paramagnetic or diamagnetic material, to estimate the response of the system. Experimental data were acquired with a custom measurement system using an excitation coil and a planar gradiometer as receiver. The output of the system for different concentration of $FeCl_3$ solutions has been used to calibrate the system. Ten in-vitro measurements of liver samples, with a volume around 50 cm^3, have been made. Measurements were compared against a biochemical iron estimation procedure and histological studies with hematoxilin-eosin and Perls Prussian blue staining. A significant agreement is observed (R=0.92, P<0.001) between biochemical and MI results. The systems and procedures used have to be adapted now for in-vivo non-invasive measurements.**

Keywords: **Magnetic induction, hemochromatosis, hepatic iron**

I. INTRODUCTION

The possibility to apply magnetic induction (MI) methods for the measurement of the electrical conductivity in poorly conducting samples ($\sigma \leq 2$ S/m) was demonstrated theoretically with analytical model [1] and experimentally by [2]. Furthermore an imaging system based on MI has been used to detect ferromagnetic particles in non-conductive materials at frequencies up to 500 kHz [3]. The possibility to apply MI techniques to characterize paramagnetic and diamagnetic materials has been proved theoretical and experimentally in [4].

An interesting biomedical application based on the characterization of magnetic properties of biological tissue is the quantification of iron overload in humans, especially in hepatic tissue. The measurement of hepatic iron overload is of particular interest in cases of hereditary hemochromatosis or in patients subject to periodic blood transfusion. In-vivo induction methods have been tried previously in animals [5] and a study of the magnetic parameters of hepatic iron stores in humans has been reported in [6], which established the value of iron concentration and magnetic susceptibility for normal human hepatic tissue. To our knowledge, only non-invasive measurement methods based on SQUID have been tested in humans [7], [8]. In these methods, a magnetometer system with a cryogenic region and a special screening room is required.

For in-vitro measurement histological studies with hematoxilin-eosin and Perls' Prussian blue and biochemical measurement are used [9]. Nuclear Magnetic Resonance has been used too for an in-vivo estimation of hepatic iron overload [10].

In this article we demonstrate the feasibility of in vitro measurement of normal (physiological) or pathological hepatic iron concentration (is possible) with our recently developed MI-system.

A theoretical model for the simulation of a cylindrical sample in the object space inside a previously developed coil-coil system is used to confirm the experimental measurement results. Additionally, a brief description of the instrumentation used is given.

II. MATERIALS AND METHODS

II.1 *Mathematical model*

Consider two coils positioned coaxially (Fig. 1.a) and spaced by a distance 2a. A sinusoidal current, of angular frequency ω, in the excitation coil produces a magnetic field B_0 that is measured at the receiver coil. Both coils are supposed to have a small radius with respect to their distance and are thus modeled as magnetic dipoles. Suppose a circular disc of radius R, thickness t (t<<2a), conductivity $\kappa = \sigma + j\,\varepsilon_0\,\varepsilon_r$, and relative permeability μ_r, placed coaxially and centrally between the coils. The magnetic field B_0 will induce eddy currents and magnetization in the disc. Eddy currents produce a perturbation ΔB_e of B_0 [1]. Moreover, a magnetic field B_d is created in the plane of the disc that magnetizes it, causing an additional perturbation ΔB_m in the sensing coil. The total relative perturbation due to this magnetization, in the sensing coil (only z component) is [4]

$$(\Delta B_m / B_0) = [a^3\, t\, R^2\, (8\, a^2 - R^2)\, (\mu_r - 1)\,] / [\,2\, \mu_r\, (a^2 + R^2)^4\,] \quad (1)$$

Above 60 K and low frequency, the complex part of the magnetic susceptibility is essentially zero for paramagnetic

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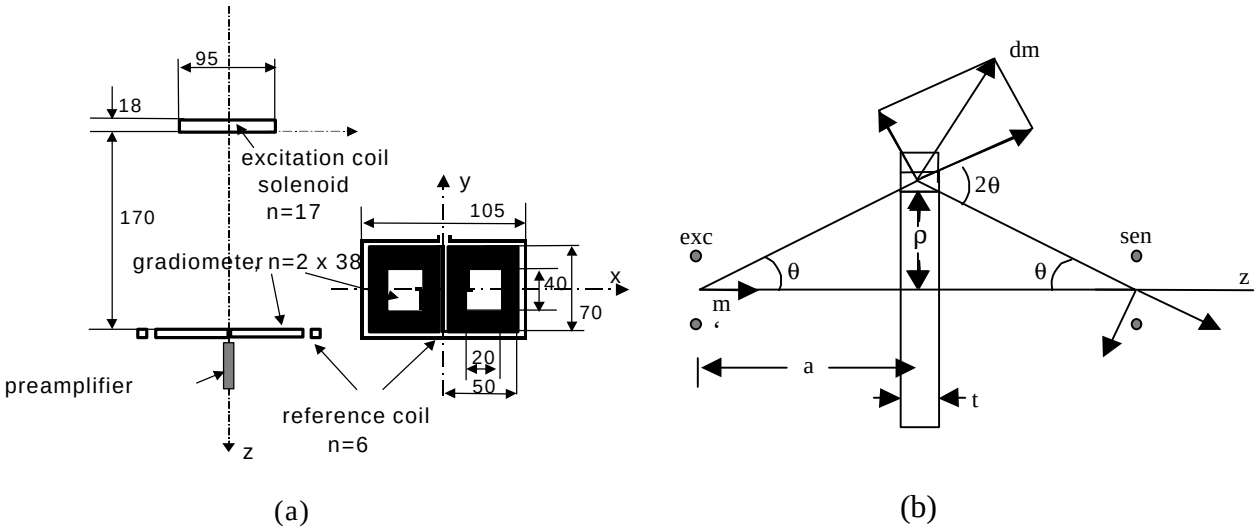

Fig. 1. (a) Geometry of the system. Excitation coil (exc) and Planar gradiometer.
(b) Paramagnetic disc inside a coaxial coil system, sen = sensor coil.

material [11]. Therefore, the complex behavior of μ_r is not considered for biological tissues at normal temperatures. The perturbation produced by a paramagnetic or diamagnetic conductor is only reflected in the real part of the total perturbation $\Delta B_t = \Delta B_e + \Delta B_m$. If we define SCR = $\Delta B_t/B_0$, Im(SCR) will essentially contain information about σ (conductivity) and Re(SCR) about the relative magnetic permeability and electrical permittivity. In diamagnetic or paramagnetic materials Re(SCR) is in the order of $\sim -10^{-6}$ for H_2O (diamagnetic) or $\sim +10^{-6}$ for 0,03 M ferric chloride solution (paramagnetic).

By definition $\mu_r = 1+\chi$ and $\chi = \mu_0 Nm^2/3KT$ (weak magnetism), where μ_0 is the permeability of the free space, N the number of magnetic dipoles per unit of volume, m the magnetic moment of each dipole, K the Boltzman constant and T the absolute temperature. Therefore Re(SCR) contains information about the relative magnetic permeability μ_r (or the susceptibility χ) and allows an estimation of concentration of magnetic dipoles.

From the practical point of view it is important to take into account all systematic measurement errors which affect Re(SCR) much more than Im(SCR), as in [12].

II.2. *Instrumentation system*

The experimental system (electronic circuits and algorithms) described in [12] has been used after adapting the data acquisition system and the excitation circuit so as to achieve maximum sensitivity. We have incorporated a board for digital coherent demodulation developed previously for electrical impedance tomography systems [13]. With this board speed was increased to 100 measurements per second. Moreover, we incorporated a new current source capable of applying 30 A_{pp} to the excitation coil. The maximum current in the excitation circuit is achieved working in serial resonance mode. The detection coil is a planar gradiometer (Fig. 1.a). The excitation coil and the PGRAD have a screening connected to ground. The preamplifier is located in the zone of low sensibility of the PGRAD, very close to it. The differential current output of the preamplifier allows signal transmission to the mainframe with high immunity to interferences.

The excitation coil and the receiver (PGRAD) were separated 17 cm, both being attached to a mechanical support made of PVC. A chopper technique was used to reduce the influence of electronic drift. The sample was moved in a plane parallel to the PGRAD, 20 times between the positive and the negative zone of maximum sensitivity of the gradiometer [14]. One hundred measurements were taken each time at each position. The real and imaginary parts of $\Delta B_t/B_0$ were measured. We used a program developed with LabVIEW (National Instruments) for automatization of the measurements and the necessary data processing, for drift correction. All samples were enclosed in plastic beakers of R = 1,75 cm and h = 6,50 cm.

Calibration was performed measuring 5 samples of $FeCl_3$ solutions. The concentrations were: 0.3, 0.1, 0.05, 0.01 and 0.002 M and the size of the sample was R=1,75 cm and t=5,00 cm.

II.3. *Measurement protocol*

We used samples of liver human cadavers coming of necropsies studies which were carried out in the Pathological Anatomic Service of the Sant Pau Hospital, Barcelona, Spain. In all cases written authorization by the family was obtained.

For MI measurements samples (between 27-68 g) were obtained less than 12 hours after death. At the same time, three small samples (few tens of mg) were taken from each liver piece and frozen for posterior biochemical analysis [15] and scoring of stainable liver iron-judged by histologic

grading with Perls' Prussian blue [9]. The output of the latter technique is a number between 1-4, with 1 meaning poor and 4 meaning strong iron content. The remainder of the sample were enclosed in a plastic beaker (R=1,75 cm and h=6,50 cm.). The weight of each sample was measured before the MI measurements at room temperature less than four hours after collecting the samples for the biochemical analysis. To detect possible malfunctions, measurements with distillate water and $FeCl_3$ (0.3 M) were made before and after the measurement of each liver sample.

II.4. *Estimation of the iron concentration*

The Re(SCR) depends on the iron concentration, mass, geometry and location of the sample in the sensibility zone. For this reason the sample was centered carefully. M_i and M_{ref} are the masses of the sample and the reference, respectively, M_{ref} being 55 g. We used distilled water as reference for zero iron concentration C_{Fe}. Re(SCR) was corrected for mass differences between different samples by the factor M_{ref}/M_i. Moreover, a factor depending of the sample volume was applied.

We obtained a calibration curve from measurements of H_2O (C_{Fe}=0) and normal liver (χ_{mH2O}= $-10*10^{-6}$ and χ_{mliver} = $-8,42*10^{-6}$ [SI]) assuming a C_{Fe}=0,25 mg/g for liver [6] The linear response of the system to changes of χ_m was tested for a group of $FeCl_3$ solutions [4]. The measured Re(SCR) was for H_2O–$1,36*10^{-6}$, for a liver sample with normal iron concentration we estimated that Re(SCR)= $-1,22*10^{-6}$.

The C_{Fe} was calculated from the measured Re(SCR) in mg/g of liver, assuming that 70% of liver tissue consists of H_2O and 30% of solids [6]. With this model and atomic mass of Fe additionally the C_{Fe} was calculated in μmol/g of dry liver tissue.

III. RESULTS

The results with all methods are shown in table 1. The correlation between iron concentration by MI and biochemical methods is shown in Fig. 2. The STD of Re(SCR) was in the order of 10^{-7}.

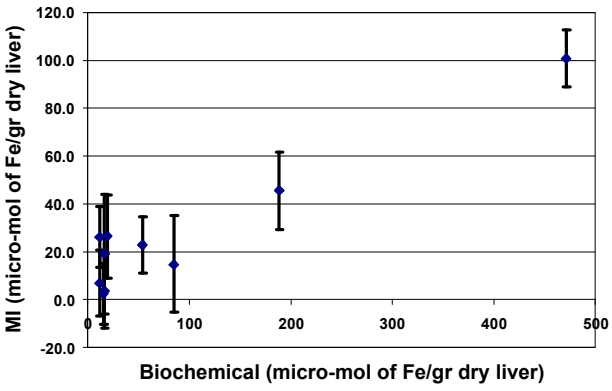

Fig. 2 Iron concentration of samples of hepatic tissue, measures for MI and biochemical methods

TABLE I.
Iron concentration using different methods.

Sample No	Mass (gr)	Re(SCR) x 10^{-6}	C_{Fe} (μmol/g liver dry) MI	C_{Fe} (μmol/g liver dry) biochem	PERLS	Histological study
M09	30,0	–1,30	4,3	16	1	Moderate-severe Esteatosis
M11	50,9	–1,29	6,9	12	1	Sligth Esteatosis
M12	47,5	+0,10	100,7	471	4	Cirrhosis. Iron in hepatocytes and macrophags
M13	43,0	–0,70	45,5	188	2-3	Macrophags iron and hepatocytes
M14	27,8	–0,98	26,1	11	1	Massive metastasis. Adenocarcinoma (small)
M15	46,0	–1,30	3,8	17	1	Non pathological liver
M16	40,0	–1,14	14,8	85	2	Macrophags iron and hepatocytes Severe colostasis.
M24	40,0	–0,80	38,2	19	2	Passive congestion
M26	62,0	–1,06	22,8	54	2	Colostasios
M33	55,5	–1,32	2,4	33	1	Moderate esteatosis

IV. DISCUSSION

The iron concentration in a normal adult liver lies around 250 μg/g or 15 μmol Fe/g dry liver (the total amount of liver iron storage will be around 400 mg) with a range of 50-500 μg/g of liver being considered normal [7].

A total iron content of more than 1 g in the liver is considered as hepatic iron overload (> 700 μg/g or 40 μmol Fe/g dry liver). Table 1 and fig. 1 shows that the system with the present method is capable of discriminate strong and medium overload cases (M12-M13).

The expected theoretical Re(SCR), using equation 1, is $-1,11*10^{-6}$ for normal hepatic tissue. The estimated value for normal liver taking into account the measurement of water and the susceptibility for water and normal liver is $-1,22*10^{-6}$, which is close to the calculated value. There is also a good agreement between measured and calculated results for distilled water.

In very strong iron overload the liver can contain up to 14 g (9 mg/g of liver tissue or 540 μmol Fe/g dry liver) [6]. Thus, the expected Re(SCR) for a 50 cm^3 sample with very strong overload, using the theoretical model, will be in the range of $+6*10^{-6}$. This value is much greater than the value measured for the sample with a comparably strong iron overload (M12). Further measurements and experiments are required to reveal the reasons for this discrepancy.

With the designed system and protocol, the STD in the Re(SCR) is in the order of $0.2*10^{-6}$. Measurement of M12 and M13 iron concentration indicate that our system is capable of resolving samples with an overload double than the pathological limit (aprox. 100 μmol Fe/g dry liver).

With a modification of the mechanical structure it will be possible to eliminate some of the errors that affect the Re(SCR) [12] and to reduce the STD, allowing an increase of the resolution.

In conclusion, the system is able to measure, with reasonable precision, high iron concentrations in hepatic tissue, presenting greater variability and inaccuracy in samples with lower metal concentration. Absolute values for iron concentration were underestimated when calculating them from the measured Re(SCR) with the theoretical model and considering published data of liver susceptibility. Consequently an experimental calibration is required. The method is promising for the non invasive measurement of the hepatic iron in humans given the bigger volume of the complete liver when compared to our samples. The mechanical aspects measurement and calibration techniques have still to be adapted to the in-vivo new situation.

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